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Acidic Buffer Induced Muscle Pain Evokes Referred Pain and Mechanical Hyperalgesia in Humans

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Abstract

While tissue acidosis causes local deep-tissue pain, its effect on referred pain and mechanical muscle hyperalgesia is unknown. The aim of this study was to investigate a human experimental acidic muscle pain model using a randomized, controlled, single-blinded study design. 72 subjects (36 female) participated in three visits, each involving one 15 min intramuscular infusion into the anterior tibialis muscle: acidic phosphate buffer (5.2 pH) at 40 ml/hr (N=69) or 20 ml/hr (N=54), normal phosphate buffer (7.3 pH) at 40 ml/hr (N=70), or isotonic saline at 40 ml/hr (N=19). Pain ratings and pressure sensitivity of superficial and deep tissues were assessed before, during, and 20 min after infusion. Acidic buffer produced light to moderate, rate-dependent, muscle pain (not sex-dependent) compared to the control infusions, that referred pain to the ankle in 80% of women and 40% of men. Pain did not vary across selfreported menstrual phases. Pressure pain thresholds (PPTs) were reduced over the infused muscle with acidic infusion, defined as primary mechanical hyperalgesia. PPTs decreased at the ankle in those with referred pain in response to acidic buffer, i.e. referred mechanical hyperalgesia, but not at the foot. No pain or changes in PPTs occurred in the contralateral leg. These results demonstrate muscle acidosis can lead to local and referred pain and hyperalgesia, with significant sex differences in development of referred pain.

Keywords

myalgia; central sensitization; experimental pain

1. Introduction

More patients seeking medical attention report musculoskeletal pain complaints than any other form of pain [28]. Although multifactorial, one known source of deep-tissue pain is tissue acidosis. Acidosis may lead to pain in muscle trigger points [51]; cardiac muscle [11]; inflammatory conditions [58]; and exercise [30,43]. Accordingly, experimental muscle pain can be induced by the intramuscular infusion of a buffered acidic solution, e.g., ascorbic acid [49] or an acidic phosphate buffer solution [35] in humans. Animal studies have revealed that

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acidic infusion models activate chemosensitive nociceptors: acid-sensing ion channels (ASICs) and/or the transient receptor potential vanilloid 1 (TRPv1), resulting in mechanical hyperalgesia [32,54,55,56].

Referred muscle pain has been consistently observed with hypertonic saline muscle pain models, but is muscle dependent. For example, stereotypic referred pain patterns occur with hypertonic saline infusion of the anterior tibialis [24] and infraspinatus [42] muscles in approximately 50% of subjects [22]. Whereas only localized pain is observed with infusion of the triceps brachii or biceps brachii muscles [22]. These distributions are consistent with referred pain patterns associated with muscle trigger points [62,63]. It is not clear whether referred pain similarly occurs with acidic pain models, or may be more clearly expressed due to activation of acid-sensitive nociceptors.

Mechanical hyperalgesia occurs with several clinical pain conditions [7], but it is not consistently observed with the experimental hypertonic saline model in humans [22]. However mechanical hyperalgesia may be stimulus specific, e.g. tissue acidosis. Animal studies demonstrate that repeated intramuscular bolus injections of acidic saline (pH 4.0) induce cutaneous and muscle mechanical hyperalgesia both ipsilateral and contralateral to the injection site [54,61]. Deep-tissue mechanical hyperalgesia has not been investigated with an acidic pain model in humans, nor are contralateral effects typically considered.

Several musculoskeletal pain conditions, such as fibromyalgia, chronic tension headache and temporomandibular joint syndrome occur more frequently in women [5,27]. However, in various experimental pain models, both greater female pain sensitivity [16,18,65,69] and no sex differences [12,41] are observed. Sex differences have yet to be investigated using an acidic muscle model in humans.

The purpose of this study was to investigate whether acid-evoked muscle pain produces referred pain and/or mechanical hyperalgesia compared to control infusions in men and women. We hypothesized 1) local and referred pain would occur in a dose-dependent manner; 2) mechanical hyperalgesia would occur as observed in animal models; and 3) women would experience greater pain and mechanical hyperalgesia than men.

2. Methods

2.1 Subjects

Seventy-two healthy, pain-free volunteers (36 male, 36 female) were recruited from the University and local community. The mean (SD) age of the participants was 24.3 ± 6.1 yrs (range 18-50 yrs). Mean ± SD height and mass were: 172.3 ± 10.4 cm and 72.4 ± 13.0 kg, respectively. The majority of the study population was non-Hispanic, Caucasian (n=59); the minority included African-American (n=6), Asian (n=5), and Hispanic Caucasian (n=2). Exclusion criteria included: current pain complaints, past history of chronic pain, significant medical history (e.g. diabetes, asthma, and heart disease), prescription medications other than birth control or vitamins, pregnancy, and history of lower extremity injury. All participants provided written-informed consent in accordance with the Helsinki Declaration prior to participation, as approved by the local Institutional Review Board. Participants were instructed that moderate muscle pain could occur, but can vary between visits and by individual, and were reimbursed for their time (~1.5 hours per visit). To monitor potential hormonal influences, women were asked to report any hormonal therapies used and the first day of their last period at each visit.

2.2 Study Protocol

A randomized, controlled, single-blinded study design was used, with each subject serving as their own control. Subjects participated in three visits, each spaced approximately 1 week apart (5-14 days). Each visit involved one 15 min intramuscular (IM) infusion into the mid-belly of the anterior tibialis muscle in a balanced-random order: acidic phosphate buffer (5.2 pH) at 40 ml/hr (acidic 40), acidic phosphate buffer (5.2 pH) at 20 ml/hr (acidic 20), and normal phosphate buffer (7.3 pH) at 40 ml/hr (PB control). After recruiting 50 participants, we chose to add a second alternative control solution, 0.9% isotonic saline at 40 ml/hr (saline control) as the PB control solution provided an acid control, but not a pain-free control (Steen et al. 1996). In the last 22 subjects recruited, we maintained a three-visit protocol, with the saline control (n=19) replacing either the acidic 20 infusion or the PB control, using a randomization process weighted towards replacing the acidic 20 infusion. Pain and sensitivity of deep and cutaneous tissues were assessed before, during, and 20 min after infusion in the local and referred pain areas and similar contralateral sites. Radial artery pulse at the wrist was measured manually every 5 min throughout the 45 min protocol.

2.3 Intramuscular Infusions

The sterile phosphate buffer and control solutions were prepared by the University of Iowa Hospital & Clinics pharmacy and stored as sterile solutions in 30 ml syringes. The acidic solution (pH 5.2, 140 mM) followed reports by Issberner et al (1996), using a 96:4 percent ratio of monosodium phosphate, monohydrate (1.89 g) to disodium phosphate, heptrahydrate (0.079 g) per 100 mL water. The acidic phosphate buffer was iso-osmotic to saline, with osmolarity measured as 283 mOsm/kg H_2O compared to 280 mOsm/kg H_2O for the 0.9% saline. No previous formulations for a control, normal pH phosphate buffer were available. Pilot studies using pH 7.2, 140 mM and pH 7.3, 50 mM phosphate buffer (0.61 or 0.185 g monosodium phosphate, 2.566 or 0.98 g disodium phosphate per 100 mL water, respectively) were both mildly painful. We chose to use the pH 7.3, 50 mM for the phosphate buffer control. The measured osmolarity for this solution was 128 mOsm/kg H_2O, thus, hypotonic. The measured sodium concentrations for each solution were: 150.7 mM (saline), 137.5 mM (5.2 pH), and 77.5 mM (7.3 pH). The isotonic 0.9% saline was not pH buffered, with samples averaging pH 5.7 ± 0.4 (SD). Despite its relative acidity, isotonic saline would be expected to have minimal effects on intramuscular pH, due to the buffering ability of the muscle.

The sterile solutions were infused at a constant rate using a syringe pump (Model A-99, Razel Scientific Instruments, USA): 40 ml/hr for the acidic 40 and controls (10 ml) or 20 ml/hr for the acidic 20 (5 ml). The infusion site was cleaned with three alcohol wipes and allowed to air dry prior to insertion of the infusion catheter (24G, 1.9 cm flexible, Medex Medical, UK). Extension tubing (94 cm, 0.2 ml, Medex, USA) connected the syringe filter to the catheter in line with a 0.22 micron filter (Millipore, Ireland), and was secured to the skin using surgical tape. The catheter remained in place the entire study protocol, and was only removed once all sensory and pain measures were completed after the 20 min recovery period.

2.4 Pain Assessment

Verbal pain reports for the local infusion site and the ankle, a common site for referred pain from the anterior tibialis muscle [22], were reported throughout the 45 min protocol using the Borg Category-Ratio 10 (CR10) scale [6]. This scale has been validated for pain [6] and provides characteristics of both a numeric pain rating scale and a category scale (e.g., 0 – no pain, 2 – light pain, 3 – moderate pain, 5 – strong pain, 10 – maximum pain) without a ceiling effect; participants can rate pain above 10 if they reach a point greater than they have ever felt or imagined before. Peak pain and pain integral (area calculated under the pain-time curve) were extracted from the verbal pain ratings. Participants completed the short form of the McGill Pain Questionnaire (SF-MPQ) [40] including: rating 11 sensory and 4 affective descriptors; a

written 10 cm visual analog scale (VAS); and a pain drawing of the lower limb approximately 10 min into the 15 min infusion.

2.5 Mechanical Sensitivity Assessment

Pressure pain thresholds (PPTs) were measured using a digital, hand-held pressure algometer (Algometer Type II, Somedic, Sweden), with a 1 cm^2, rubber-tipped probe at a rate of 30 kPa/s. Participants were instructed to press the hand-held trigger when the pressure *first* became painful, "approximately a 1 on the Borg 0 to 10 pain scale." PPTs were determined at 4 locations ipsilateral to the infusion (upper and lower anterior tibialis, anterior ankle, and web space between $1^{st} - 2^{nd}$ metatarsal on the foot) and 2 mirrored contralateral locations (lower anterior tibialis and ankle). See figure 5F for a schematic drawing of sensory testing locations. Six pre-determined, randomly-ordered testing patterns were used to test PPTs (12 subjects selected at random per pattern) to minimize possible order effects. One round of training PPTs were performed and discarded prior to the baseline measures. The mean of two repetitions were assessed for each site six times throughout the protocol; two baseline (pre- and post-catheter insertion prior to infusion); two during infusion (5 and 13 min after initiation); and two recovery (7 and 20 min post infusion). PPTs were normalized by baseline values; deep-tissue mechanical hyperalgesia was operationally defined as a lower pressure pain threshold relative to baseline (e.g. less than 100%).

Cutaneous sensation was measured at the same six sensory test locations using a single 100g von Frey filament (Touch Test 6.10, North Coast Medical, Inc., USA). Following three perpendicular stimulations to the skin surface at a rate of ~1 Hz, subjects indicated the intensity of the sensation on a written, 10 cm VAS. The scale was anchored with 0 = no sensation (completely unaware of the filament touch), 5 = pain threshold, and 10 = maximum pain. The same pattern of testing was used for PPT and von Frey filament stimulation. However, only 5 sets of testing were performed, 2 baseline, 1 during infusion (7 min after initiation), and 2 during recovery (4 and 19 min post infusion).

2.6 Statistical Analysis

Descriptive statistics (mean, SEM) were calculated for all pain and sensory variables. Frequency analyses (Chi-square, Fisher's exact test, contingency tables) were performed to assess if differences in the incidence of local or referred pain occurred between infusions. Presence of local or referred pain was operationally defined as peak pain ≥ 0.5 ("just barely noticeable") on the Borg CR-10 scale. Associations between pain ratings: local and referred verbal ratings and the McGill VAS, were assessed using Spearman's rank correlation coefficient. Normality of the pain and sensory variables was assessed using the Kolmorgorov-Smirnov test. When required, a natural log transformation, $X' = \ln(X + 1)$, was applied to achieve normality prior to the remaining statistical procedures.

Two-way mixed, repeated measures analyses of variance (ANOVA) using a general linear model were used to test for differences in pain variables between infusions and by sex. Differences in PPT and von Frey scores relative to baseline were assessed using three-way mixed, repeated measures ANOVA (infusion by time by sex) for each sensory test site. The Huynh-Feldt correction was applied as necessary to correct for non-sphericity resulting from the repeated measures design [31]. Post-hoc tests utilized paired or independent t-tests as appropriate. To examine the influence of menstrual cycle on local and referred pain, ANOVA was used to compare peak pain values between 3 menstrual cycle phases. These phases were operationally defined as: the menstrual/ follicular phase (days 1 – 10), ovulatory (days 11-17), and luteal phase (days 18-28) based on reported patterns of estradiol, progesterone, and luteinizing hormone in women [60]. Significance was set at $p \leq 0.05$.

3. Results

Sixty-nine of the 72 enrolled participants completed all three visits of the study. One participant sprained his ankle and therefore no longer met the inclusion criteria; the other two chose not to complete the study. Total sample size for each infusion included: 69 (35 F) for the acidic 40; 54 (27 F) for the acidic 20; 70 (36 F) for the PB control; and 19 (8 F) for the saline control. Of the 36 females, 17 reported taking oral hormonal therapy, 1 was post-menopausal, and 1 was more than 1 year post-partum without a menstrual cycle. No complications occurred during the study, and heart rate did not vary during any of the infusions.

3.1 Local Pain

The acidic infusion model produced muscle pain quality was most frequently described on the McGill Pain Questionnaire (MPQ) as aching, throbbing, cramping and tender across all infusions. Peak pain ratings were not distributed normally, but were positively skewed, thus were log transformed. For clarity, however, original pain scores are reported here. Mean (SEM) peak pain ratings were higher for the acidic 40 (3.0 ± 0.2) than the acidic 20 (2.1 ± 0.2; $p < 0.001$), PB control (2.4 ± 0.2; $p \leq 0.009$), or saline control (0.9 ± 0.3; $p \leq 0.001$) infusions. The muscle pain developed steadily over the first 3 min, maintaining a relatively constant, ratedependent intensity during the remainder of the infusion (figure 1). Representative pain drawings for eight participants experiencing each infusion are shown in figure 2. When considering the area under the curve for the pain ratings (pain-time integral), the acidic 40 infusion produced the greatest pain ratings, similar ratings were reported for the acidic 20 and PB control infusions, with the lowest ratings for the saline control infusion (figure 3A). More participants reported local pain (rating ≥ 0.5 on Borg CR10) during the acidic 40 infusion (100%) than during the acidic 20 (89%, $p = 0.006$) or the saline control (74%, $p < 0.001$) infusions, but not compared to the PB control (94%, $p = 0.06$). No sex-differences or testing order effects were observed for local pain ratings across infusions. Peak local pain ratings did not significantly vary between the three menstrual phases across infusions ($p = 0.09$ to 0.54).

Individuals reported their pain experience similarly using different methods. Pain ratings were positively correlated between the peak verbal infusion site pain ratings (Borg CR10) and the single overall VAS pain rating on the MPQ for each infusion (range: $r = 0.53$ to 0.70, $p = 0.02$ to $p < 0.0001$). Individuals with higher infusion site pain during one infusion also reported higher pain with other infusions (see table 1).

3.2 Referred Pain

Referred pain was reported only at the ankle, developing over approximately 4 min, and maintaining a constant intensity during the 15 min infusion (figure 1). Referred pain (≥ 0.5) occurred more frequently during the acidic 40 (62%) than the saline (37%) infusions ($p < 0.05$), but was not significantly different than the acidic 20 (48%) or PB control (54%) infusions. Significantly more women than men experienced referred pain during the acidic 40 (80% vs. 44%, $p < 0.05$), acidic 20 (63% vs. 33%, $p < 0.05$), and PB control (69% vs. 37%, $p < 0.05$) infusions, respectively. No sex-differences in referred pain were observed for the saline infusion. However, peak referred pain did not vary with menstrual phase across infusions ($p = 0.19$ to 0.58).

Peak referred pain and the area under the referred pain curve (pain-time integral, figure 3A) were greater for the acidic 40 than the remaining three infusions ($p < 0.001$) across all subjects. When considering only those individuals with referred pain, peak referred pain intensity (mean Borg CR10, SEM) remained significantly greater for the acidic 40: 1.8 (0.3), than the acidic 20, PB control, and saline infusions: 1.4 (0.2), 1.2 (0.1), and 0.9 (0.1), respectively ($p < 0.01$). Thus, the difference in referred pain intensity between the acidic 40 versus the other conditions

was maintained and not merely a result of the difference in referred pain incidence. Women had significantly greater referred pain intensities than men for the acidic 40, acidic 20, and PB control infusions (figure 3C, $p < 0.05$).

Referred pain intensity was moderately associated with local pain intensity during the acidic 40, acidic 20, and PB control infusions (figure 4, $p < 0.001$), but not during the saline control infusion. However, these relationships were largely driven by stronger associations in women than men. Peak local – referred pain correlations were typically high in women: 0.73 ($p < 0.0001$), 0.66 ($p < 0.0001$), 0.56 ($p < 0.0001$) and 0.40 ($p = 0.33$); whereas in men the correlations were relatively low: 0.13 ($p = 0.48$), 0.50 ($p = 0.008$), 0.29 ($p = 0.10$), and -0.07 ($p = 0.85$) for the acidic 40, acidic 20, PB control and saline infusions, respectively. Referred pain ratings between infusions revealed significant correlations between all combinations of the acidic 40, acidic 20 and PB control infusions (table 1).

3.3 Sensory Testing

PPTs normalized by their baseline values varied between infusions ($p = 0.02$) and across time ($p < 0.001$) for the upper and lower anterior tibialis muscle test locations. Post-hoc tests revealed significant decreases in PPT during the two acidic infusions when compared to either control infusions (figure 5A, $p \leq 0.01$). PPTs remained significantly decreased seven min following infusion, but returned to baseline by 20 min after infusion. There were no differences between the upper and lower anterior tibialis sites, thus these data were collapsed for illustrative purposes. No sex-differences were observed in the normalized PPT measures, however absolute PPTs were significantly lower in women than men at all test locations ($p < 0.0001$).

Normalized PPTs at the ipsilateral and contralateral ankle and contralateral anterior tibialis sites varied across time ($p < 0.0001$) but not between infusions (figure 5 B, D, E). Post hoc tests revealed small but significant decreases in PPT over the ipsilateral ankle and increases in pressure pain thresholds contralaterally during infusions ($p < 0.05$). There were no changes in PPTs at the web space of the foot (figure 5C). The ipsilateral ankle, i.e. the referred pain site, was the only location to demonstrate a significant influence of sex (time - sex interaction, $p = 0.008$). Post-hoc tests revealed this apparent sex difference at the ankle covaried with the sex difference in referred pain incidence. In individuals with referred pain, PPTs were significantly lower than in those without referred pain at both the infusion site ($p = 0.02$) and the ankle ($p = 0.01$) during the acidic 40 infusion (figure 6A).

Mean sensory ratings during cutaneous von Frey testing were below the pain threshold (i.e., 5 cm) at all test locations (range 1.8 – 2.4 cm). Although the ANOVA resulted in significant increases in sensory ratings across time at the contralateral ankle ($p = 0.006$) and lower anterior tibialis ($p = 0.01$); post hoc tests revealed no significant pair wise differences between infusions at each time or between test times for each infusion. The largest mean increase in sensory ratings during von Frey filament testing (more sensitive) was 0.35 cm at the ankle during the acidic 40 condition.

4. Discussion

Acidic muscle pain evokes rate-dependent local pain and referred pain, with ipsilateral deep mechanical hyperalgesia, but no contralateral pain or mechanical hyperalgesia. Sex differences were observed for select pain measures: with more females experiencing referred pain, females exhibiting a stronger correlation between local and referred pain, and lower baseline PPTs in females.

4.1 Local and Referred Pain

As hypothesized, a rate-dependent pain response was observed, with peak local and referred pain more intense for the higher rate infusion, consistent with previous reports [35]. Similar referred muscle pain patterns and overall incidence were produced with the acidic and the hypertonic saline models [22,23,24]. Distinct referred pain occured only at the ankle (figure 1C), not merely an enlargement of the local pain region. However, the acidic buffer infusion produced relatively stable pain, whereas constant-rate hypertonic saline models typically produce an initial peak with a gradual decay over time [22].

Several theories have been proposed to explain the referred pain phenomenon, with the convergence-projection theory the most widespread. Input from different tissue types (i.e. muscle, viscera, skin) converge on the same dorsal horn neurons [70]. After injury, increased nociceptive input from the injured muscle, for example, is transmitted supraspinally and misinterpreted at the cortical level as pain from other tissues. However, this theory in isolation does not explain referred pain directionality, e.g. cardiac pain refers to the shoulder, but the reverse is uncommon. Central changes are likely involved as well [1,20,22]. Dorsal horn neurons sensitize after injury resulting in increased receptive field size that would further contribute to the referred pain area. In fact, intramuscular injection of the inflammatory irritant, bradykinin, results in newly developed receptive fields of dorsal horn neurons in rats [29]. These authors conclude that pathways exist to produce referred pain and hyperalgesia but aren't functional until the appropriate nociceptive stimulation is present. Indeed, intramuscular injections of acid result in widespread hyperalgesia with sensitization of dorsal horn neurons and activation of supraspinal pathways [55,61]. Similarly, in numerous patient populations referred pain areas are enlarged in response to hypertonic saline infusions [3,25,37,42,53,59], supporting that central sensitization involving spinal and supraspinal pathways is involved in referred pain.

It is not clear why referred pain is observed in only a portion of the population. The incidence of referred pain following infusion of acidic buffer (60%) is consistent with hypertonic saline models [22]. With the acidic model, women develop referred pain more frequently (80%) than men (40%). Further, referred and local pain intensities were associated in women only. Although not always investigated, similar sex-differences in referred pain incidence occur with hypertonic saline, 67.4% versus 37.5%, and electrically-induced muscle pain, 32.3% versus 7.7%, for women and men respectively, despite no differences in local pain [21]. An acidic-evoked esophageal pain model results in greater referred pain in females [46]. However, contrary to our findings, men exhibited greater esophageal mechanical hyperalgesia.

The mechanisms underlying sex differences in referred pain prevalence are not clear. The pain does not appear to vary across the menstrual cycle, consistent with a recent review of the literature [52]. Response bias or gender-role expectations [47] may be a factor; however, local infusion-site pain and mechanical hyperalgesia did not differ between sexes. Sex differences in referred pain may be a result of spinal or supraspinal mechanisms. Temporal summation, also believed to be centrally-mediated, typically occurs at a higher rate in women in response to thermal [13,19,48,50] and mechanical stimuli [50]. However, the activation of supraspinal pain modulation systems, such as diffuse noxious inhibitory controls (DNIC), do not generally differ between men and women [2,14,16,45]. Although one study observed greater DNIC in males than females [16]. Clinically, many chronic musculoskeletal pain conditions have a female predominance, i.e. fibromyalgia, temporalmandibular disorder, chronic fatigue syndrome, arthritis [5,27]; thus the enhanced likelihood for development of referred pain in females may provide an underlying explanation for this phenomenon. However, the mechanisms remain elusive.

4.2 Mechanical Hyperalgesia

The mechanical hyperalgesia observed with this acidic infusion model is consistent with cutaneous acidic models, producing mechanical hyperalgesia to von Frey stimulation [57]. However, it has not been typically observed with other deep-tissue pain models, including intramuscular electrical stimulation [38] and hypertonic saline injection [17,22,26]. Accordingly, the acidic infusion may provide a means to study mechanical hyperalgesia not readily available with these other models.

Referred pain and mechanical hyperalgesia is likely a result of central mechanisms; while local hyperalgesia is largely peripherally mediated [70]. Nevertheless, referred hyperalgesia and central sensitization can clearly be driven by peripheral nociceptive sensitization after injury, requiring initial input from nociceptors. As mentioned above, sensitization of dorsal horn neurons with expansion of receptive fields could underlie the referred pain and hyperalgesia [29,55]. These spinal changes could be driven by supraspinal sites since blockade of brainstem sites not only reduces hyperalgesia but also reduces spinal release of the excitatory neurotransmitter glutamate after tissue injury [15,44,61].

Clinically, mechanical hyperalgesia occurs in referred pain regions in patients with musculoskeletal [39] or visceral [66] pain origins. A prolonged pain experience may increase the likelihood of developing referred pain [22], partially explaining the discrepancy often observed between experimental and clinical pain conditions, but may also be dependent on the underlying algesic stimulus. Thus, mechanical hyperalgesia in the referred pain region may be associated with the local activation of specific acid-sensitive nociceptors, and not readily induced via hypertonic saline or electrical stimulation models.

4.3 Acidic Pain and Hyperalgesia

The acidic solution produces the greatest pain response, suggesting proton-activated nociceptors are involved. However, the PB control infusion produces equivalent pain to the lower-rate acidic 20 infusion, possibly a result of the hypotonicity of the solution, or differences in Ca^{2+} chelation between solutions. ASIC activation by protons competes with Ca^{2+}, so that changes in extracellular Ca^{2+} from the infusion can alter channel kinetics [33]. Thus, the pain associated with the PB control infusion may be a result of different nociceptive activation than the acidic infusion. The isotonic saline infusion produces minimal pain that began to decay prior to the end of the infusion, suggesting limited mechanical sources of pain. Thus, the mechanisms contributing to the deep tissue pain in this model are largely chemo-mediated: primarily proton activation, possibly Ca^{2+} mediated, and minimally from tissue distension.

Nociception resulting from the acidotic environment is likely due to activation of ASICs and/or TRPV1. ASICs (ASIC1, ASIC2 and ASIC3) are located in the periphery and in dorsal root ganglion innervating muscle [67]. ASIC currents are transient, but have varying time constants, with the ASIC1a and ASIC2a maintaining depolarization longer than the ASIC1b or ASIC3 subunits [4,67,68]. It is not entirely clear how transient ASIC currents mediate sustained pain when exposed to a prolonged acidic environment. However, in addition to rapid ASIC depolarization through H+ binding, ASICs may be activated by the unbinding of Ca^{2+}, resulting in shallow, sustained currents [34]. Pain recovery following the acidic infusion appears to have two-phases: an initial rapid decay over the first 4 -5 min, followed by a slower, prolonged decay (figure 1), when compared to controls. A two-phase decay may be a result of channel kinetics of two (or more) ASIC subunits or the ASIC binding interactions between H^+ and Ca^{2+}.

Peripherally-located ASICs are critical for the development of mechanical hyperalgesia with deep tissue insult. Specifically, secondary mechanical hyperalgesia does not develop in ASIC3 knockout mice [32,55,56]. Re-expression of ASIC3 in muscle from ASIC3-/- mice restores

the development of mechanical hyperalgesia that normally occurs after muscle injury [54]. In animals, intramuscular acid injections result in contralateral mechanical hyperalgesia after two injections, but not after one [54]. No contralateral hyperalgesia was observed in this model, possibly due to: solution volume, pH or buffering capacity, number of injections, and species.

The second candidate for nociception in this pain model is a polymodal receptor, the TRPV1 channel, located on peripheral sensory neurons and activated by capsaicin, protons, heat and endovanilloids [10]. TRPV1 demonstrates pH responsiveness [9], but may not be a primary mediator of acid-evoked pain. Rather, it is sensitized by protons resulting in heightened responses to additional nociceptive stimuli [8,36]. This model (pH 5.2) may involve TRPV1 as capsazepine partially blocks cutaneous acidic pain in human subjects using pH 5.0 but not pH 6.0 [64]. TRPV1 is most noted for its chemical and thermal sensitivity, whereas mechanical sensitivity, consistent with our experimental observations, is more typically associated with ASICs.

In summary, this acidic experimental pain model provides a temporary light to moderate muscle pain that reproduces the common experience of deep-tissue pain in humans. Similar to the more common hypertonic saline model, it produces referred pain at the ankle, but additionally can produce local and referred mechanical hyperalgesia, without the need to increase infusion rate to produce a constant pain. Women experienced referred pain more than men, providing evidence of sex-dependent central sensitization, but without compelling evidence for specific hormonal effects. Future studies are warranted to further investigate the underlying mechanisms of the observed sex differences and factors contributing to the occurrence of referred pain and hyperalgesia.

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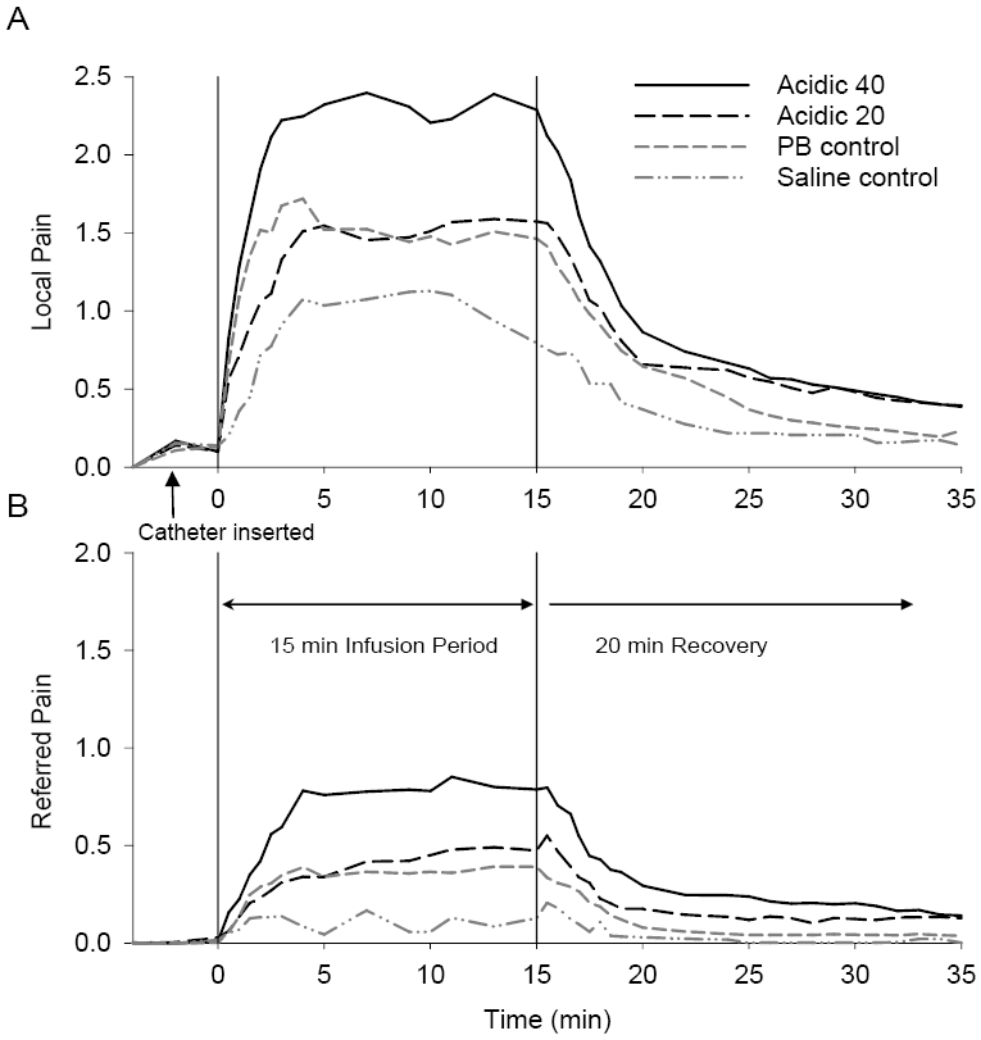

Figure 1.

Mean pain ratings (Borg CR10 scale) across all subjects at: A) the local infusion site (anterior tibialis muscle) and B) the referred pain site (ankle) including baseline (- 4 min), catheter insertion (-2 min), infusion period (0 – 15 min), and recovery (15 – 35 min) for each infusion: acidic phosphate buffer, pH 5.2, 40 ml/hr (Acidic 40; N=69); acidic phosphate buffer, pH 5.2, 20 ml/hr (Acidic 20, N=52); normal phosphate buffer, pH 7.3, 40 ml/hr (PB control, N=71); and 0.9% saline, 40 ml/hr (saline control, N=19).

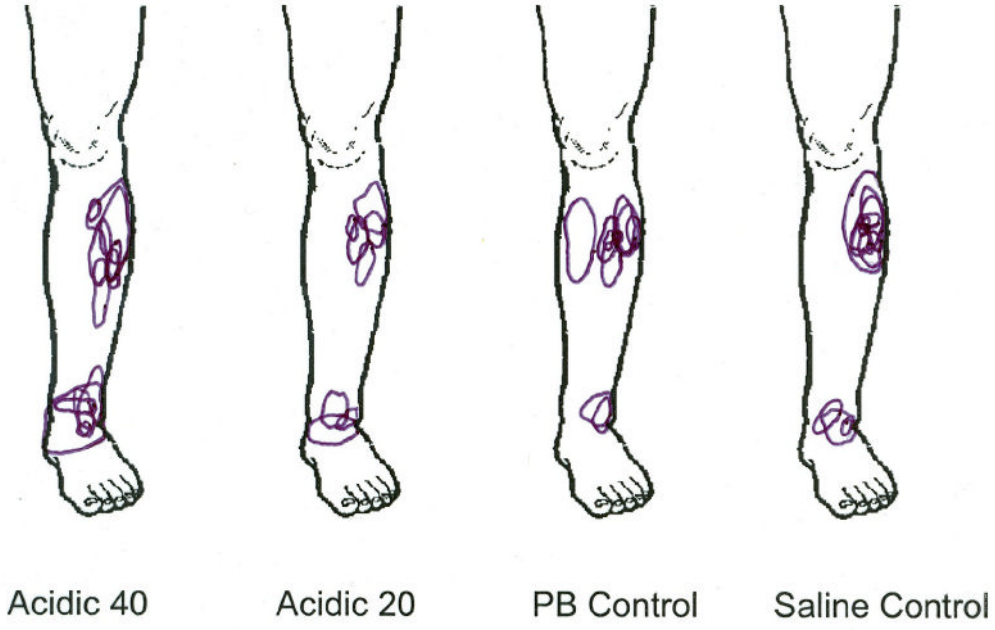

Figure 2.
Representative examples from eight individuals (4F:4M) of pain drawings of local and referred pain during each 15 min infusion: acidic phosphate buffer, pH 5.2, 40 ml/hr (acidic 40); acidic phosphate buffer, pH 5.2, 20 ml/hr (acidic 20); normal phosphate buffer, pH 7.3, 40 ml/hr (PB control); and 0.9% saline, 40 ml/hr (saline).

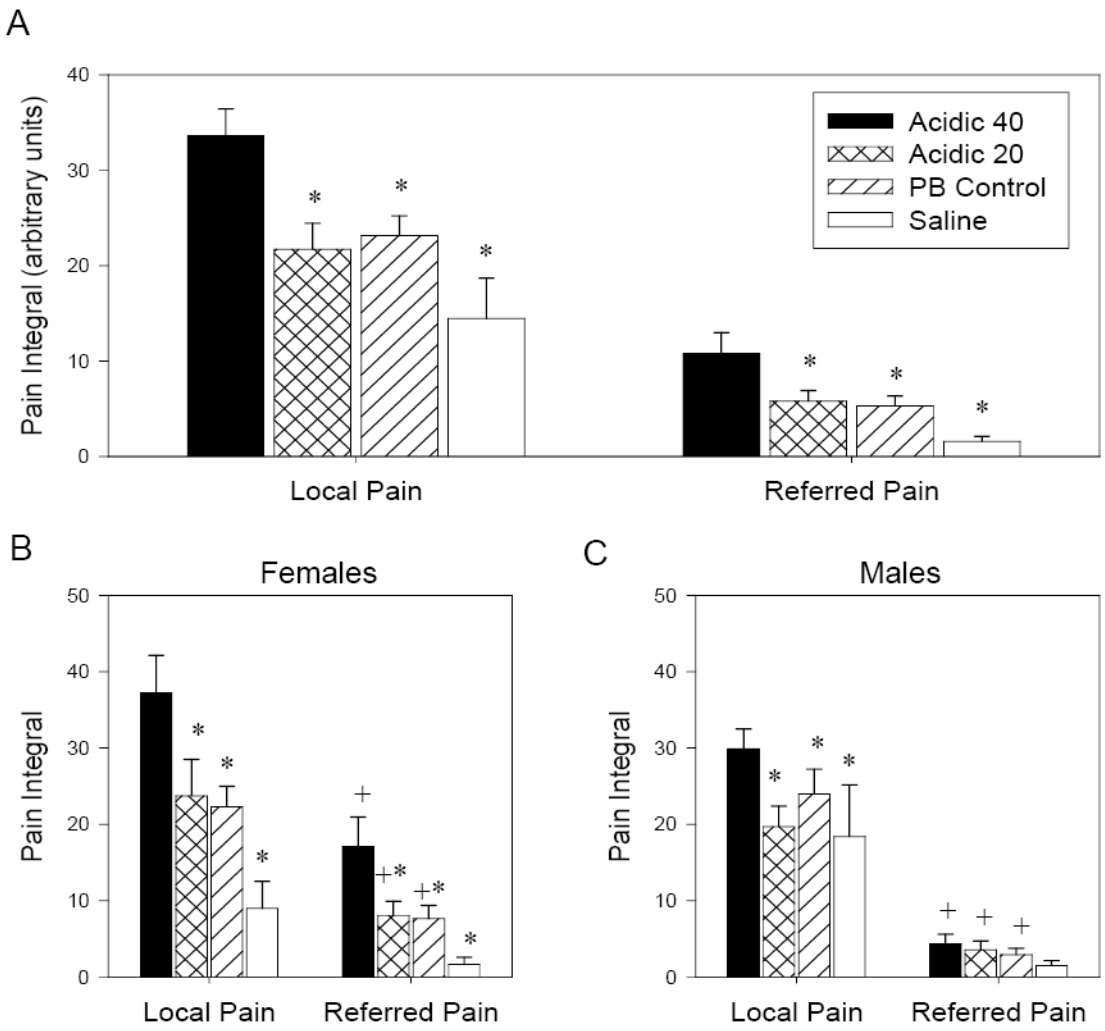

Figure 3.

Mean (SEM) pain-time integral (area under the pain-time curve) for A) all subjects by infusion for the anterior tibialis muscle (local site) and ankle (referred site) during the 15 minute intramuscular infusion; B) females only and C) males only. Infusions include: acidic phosphate buffer, pH 5.2, 40 ml/hr (acidic 40); acidic phosphate buffer, pH 5.2, 20 ml/hr (acidic 20); normal phosphate buffer, pH 7.3, 40 ml/hr (PB control); and 0.9% saline, 40 ml/hr (saline). * indicates significant ($p < 0.05$) difference from Acidic 40 infusion; + indicates significant ($p < 0.05$) difference between males and females.

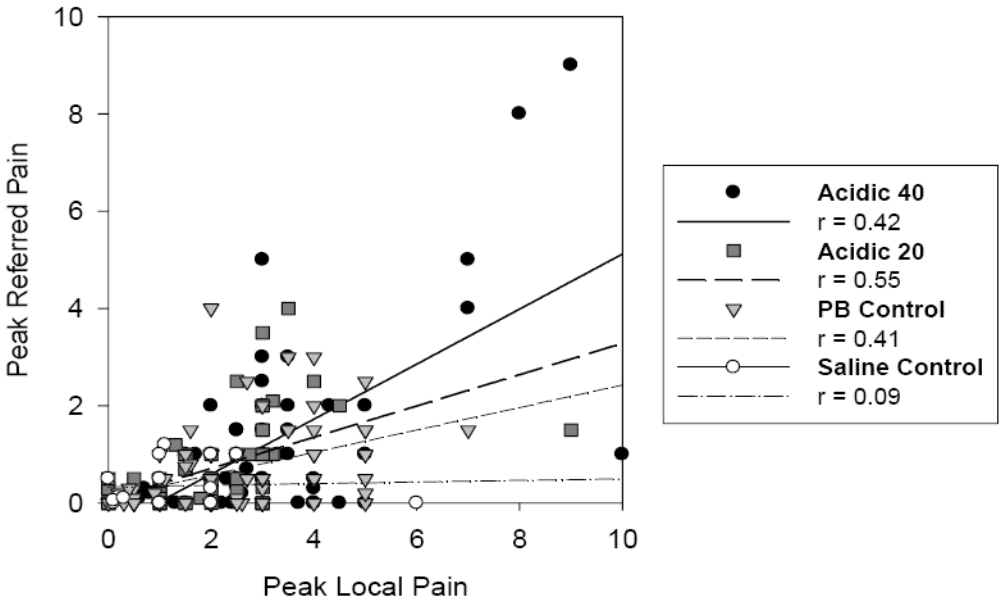

Figure 4.

Associations (r, Spearman's rho) and regression lines between peak referred pain and peak local pain for each infusion: acidic phosphate buffer, pH 5.2, 40 ml/hr (acidic 40, black circle); acidic phosphate buffer, pH 5.2, 20 ml/hr (acidic 20, gray square); normal phosphate buffer, pH 7.3, 40 ml/hr (PB control, gray triangle); and 0.9% saline, 40 ml/hr (saline control, white circle). Referred pain was moderately correlated to local pain across all but the saline infusion.

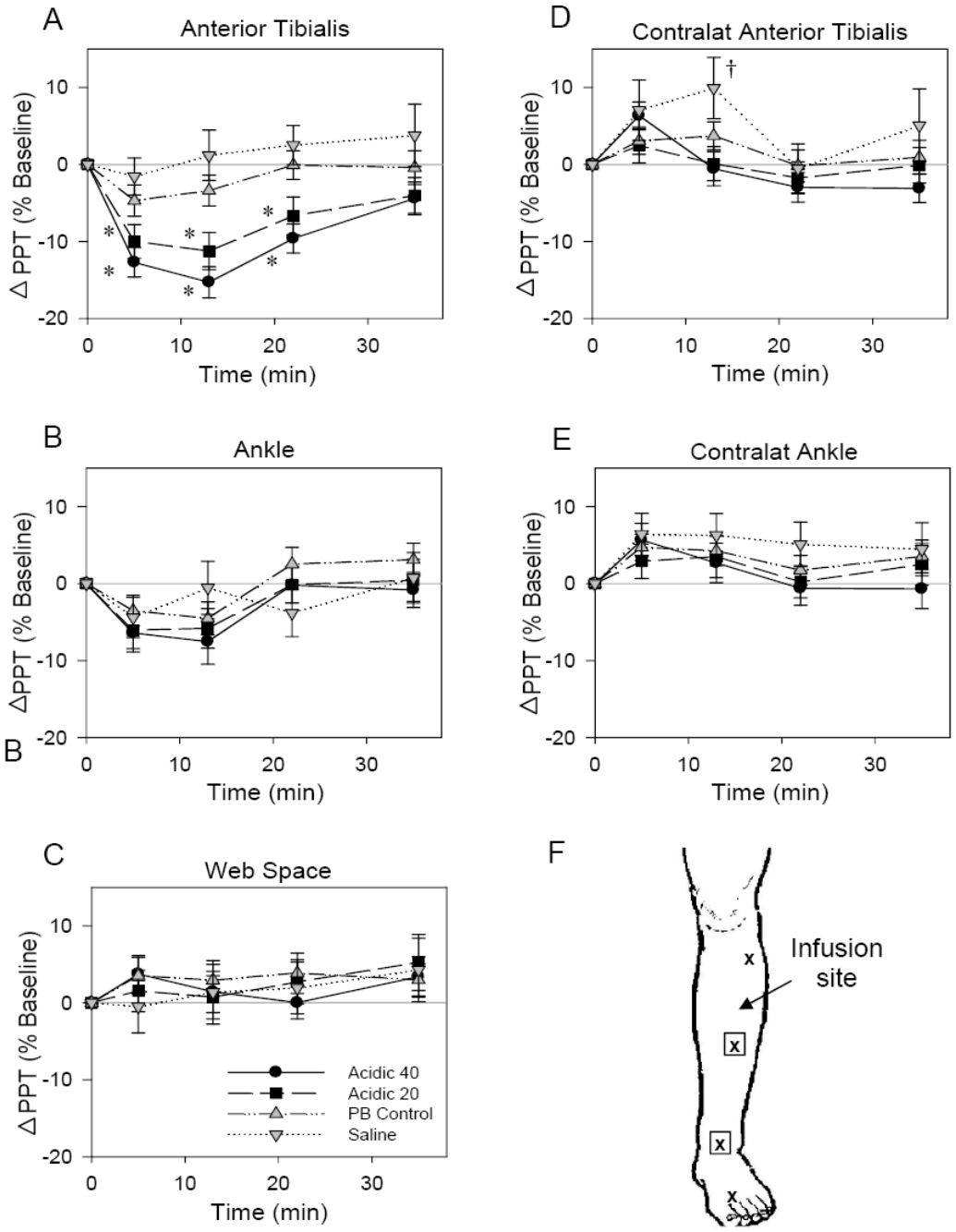

Figure 5.

Mechanical pressure pain thresholds (PPTs) relative to baseline pre-infusion values at A) mean of upper and lower anterior tibialis muscle sites, B) ipsilateral ankle (referred pain site), C) ipsilateral 1st and 2nd metatarsal web space, D) contralateral lower anterior tibialis muscle, and E) contralateral ankle; with F) sensory test locations indicated as x = ipsilateral and □ = contralateral test sites. Negative values indicate mechanical hyperalgesia. * Significantly less than control infusions ($p \leq 0.05$). † Significantly greater than acidic 40 infusion ($p \leq 0.05$).

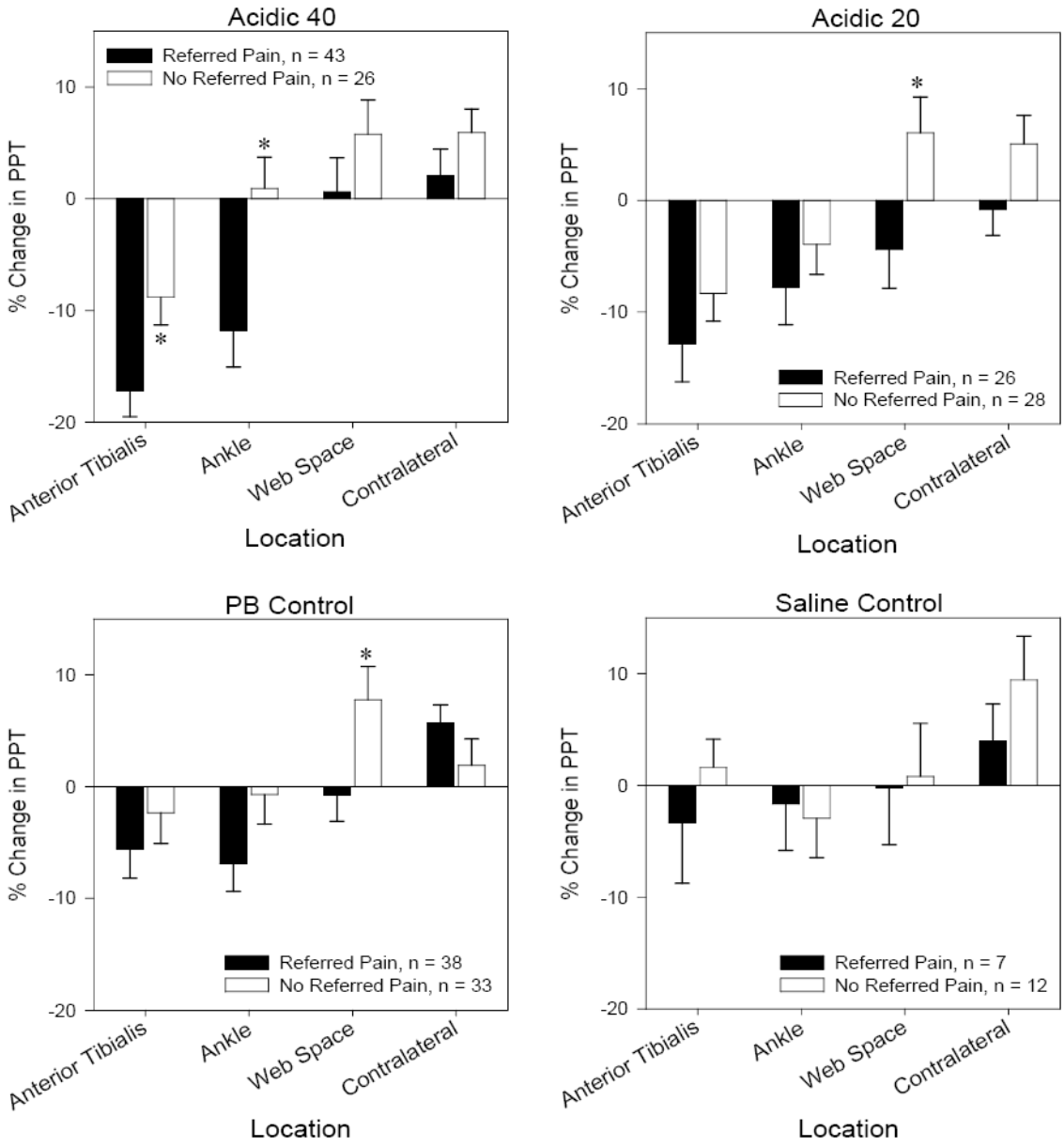

Figure 6.

Mean (SEM) percent change in PPTs relative to baseline, comparing those with referred pain (≥ 0.5 ankle pain, black) to those without (white) during each infusion: A) acidic phosphate buffer, pH 5.2, 40 ml/hr (acidic 40); B) acidic phosphate buffer, pH 5.2, 20 ml/hr (acidic 20); C) normal phosphate buffer, pH 7.3, 40 ml/hr (PB control); and D) 0.9% saline, 40 ml/hr (saline). The six test sites were collapsed to four for illustrative purposes: Anterior Tibialis (upper and lower anterior tibialis); ankle (referred site); web space (between 1st and 2nd metatarsals); and contralateral (ankle and lower anterior tibialis locations). * Significant between group differences ($p \leq 0.05$); negative values indicate mechanical hyperalgesia.

Table 1

Peak pain correlation coefficients (Spearman rho) and corresponding sample size (n) between infusions for the local infusion site (above diagonal) and referred ankle site (below diagonal).

	Acidic 40	Acidic 20	PB control	Saline
Acidic 40		0.62** 53	0.55** 64	0.25 18
Acidic 20	0.73** 53		0.37* 54	0.26 8
PB control	0.51** 68	0.63** 54		0.65* 19
Saline	0.13 18	0.39 8	0.03 19	

* $p \leq 0.01$

** $p < 0.0001$